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Ecological Processes Shaping Bulk Soil and Rhizosphere Microbiome Assembly in a Long-Term Amazon Forest-to-Agriculture Conversion

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Abstract
Forest-to-agriculture conversion has been identified as a major threat to soil biodiversity and soil processes resilience, although the
consequences of long-term land use change to microbial community assembly and ecological processes have been often neglected. Here, we combined metagenomic approach with a large environmental dataset, to (i) identify the microbial assembly patterns and,(ii) to evaluate the ecological processes governing microbial assembly, in bulk soil and soybean rhizosphere, along a long-term forest-to-agriculture conversion chronosequence, in Eastern Amazon. We hypothesized that (i) microbial communities in bulk soil and rhizosphere have different assembly patterns and (ii) the weight of the four ecological processes governing assembly differs between bulk soil and rhizosphere and along the chronosequence in the same fraction.
Community assembly in bulk soil fitted most the zero-sum multinomial (ZSM) neutral-based model, regardless of time. Low to intermediate dispersal was observed. Decreasing influence of abiotic factors was counterbalanced by increasing influence of biotic factors, as the chronosequence advanced. Undominated ecological processes of dispersal limitation and variable selection governing community assembly were observed in this soil fraction. For soybean rhizosphere, community assembly fitted most the lognormal niche-based model in all chronosequence areas. High dispersal and an increasing influence of abiotic factors coupled with a decreasing influence of biotic factors were found along the chronosequence. Thus, we found a dominant role of dispersal process governing microbial assembly with a secondary effect of homogeneous selection process, mainly driven by decreasing aluminum and increased cations saturation in soil solution, due to long-term no-till cropping.
Together, our results indicate that long-term no-till lead community abundances in bulk soil to be in a transient and conditional state, while for soybean rhizosphere, community abundances reach a periodic and permanent distribution state. Dominant dispersal process in rhizosphere, coupled with homogeneous selection, brings evidences that soybean root system selects microbial taxa via trade-offs in order to keep functional resilience of soil processes.

Keywords Metagenomics · Microbial dispersal · Neutral theory · Selection · Soybean rhizosphere

Introduction

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Forest-to-agriculture conversion often leads to loss of soil mi- crobial diversity and biotic homogenization [1–3]. Otherwise, it is widely known that long-term no-till ameliorate soil phys- icochemical characteristics, such as soil structure, porosity, organic carbon, and nutrient availability [4]. What is yet to know is whether those benefits for physicochemical parame- ters reflect in changes in microbial community assembly and if those communities can reach a new stable state [5] after soil disturbance caused by deforestation [6]. Some studies have raised the hypothesis that, in spite of a general loss of soil biodiversity due forest-to-agriculture conversion, plants can select a specific microbiome in the rhizosphere, in order to guarantee the functional resilience in the soil ecosystem [7, 8]. Thus, microbial community structure and composition in

rhizosphere can differ from bulk soil, which may result in divergent assembly patterns [9].

The emphasis in microbial ecology is mostly on the diver- sity, structure, and composition patterns rather than processes governing community assembly and diversification process [10–12]. Most of the studies connecting those patterns and processes are, in general, based on selection, which is recog- nized as an important process modulating microbial commu- nity evolution and diversification [13, 14]. Selection is evalu- ated through deterministic interactions among sets of popula- tions in a certain community and between the community and environmental factors that can modulate diversification pro- cess, composition, and abundance of species in a certain eco- system [15–17]. In summary, selection is a result of biotic and abiotic effects that combined determines fitness differences among species and individuals from the same species. The outcome of interactions between biotic (e.g., plant genotype) and abiotic (e.g., soil properties) can result in variable or ho- mogeneous selection [18]. Most of the ecological models based on selection were primarily developed for interactions among two or a few species [19, 20]. The challenge in work- ing with complex and species-rich environments, such as soils, lies in the fact that the possible assembly configurations among species or sets of populations are almost limitless [12, 21]. Those differences in microbial structure and composition in rhizosphere of several plants could be a result of selection process, modulated by the plant root system [9, 22–24].

Although selection could explain, in a certain way, the differences in microbial community assembly and diversifica- tion, empirical and theoretical models have shown that that not only selection but also drift and dispersal processes could be interacting with diversification [14], resulting in differential assembly patterns across soil types, soil fractions, time, and host plant species [25–28]. However, those processes have been often neglected in ecology studies. The variation in mi- crobial assembly related to spatial distance between sites, apart from environment influence, is an indicative of drift process. Furthermore, drift can be interpreted as part of that residual variation, unexplained by the model. Drift is referred as the dominant ecological process when residual variation is higher than the explained variation (R^2 residue > 50%) [14]. Dispersal refers to the predisposition of individuals of certain community to migrate [29, 30]. It can interact with selection and drift at the regional and local scales, modulating microbial dynamics [14]. Recently, emphasis has been given to the role of these processes on shaping microbial communities in dif- ferent ecosystems [13, 18, 25], such as agricultural soils [27] and the soil-rhizosphere interface [7]. Evaluating microbial community assembly at the light of ecological processes could make it easier to have a comprehensive picture about the boundless amount of patterns arrangements [31] and the inter- play between deterministic and neutral processes governing those patterns [32, 33]. In this study, we hypothesized that (i)

microbial communities in bulk soil and rhizosphere have dif- ferent assembly patterns and (ii) the weight of the four eco- logical processes governing assembly differs between bulk soil and rhizosphere and along the chronosequence in the same fraction. Then, we aimed (i) to identify the microbial assembly patterns and, (ii) to evaluate the ecological processes governing microbial assembly, in bulk soil and soybean rhi- zosphere, along a long-term forest-to-agriculture conversion chronosequence, in Eastern Amazon.

Material and Methods

Soil Sampling, Mesocosms Experiment, and Environmental Analyses

The dataset used here is the same as in [34], such that we provide a brief summary of methods used to generate those data. In order to evaluate long-term effects of forest-to- agriculture conversion on microbial assembly and ecological process, we analyzed bulk soil and soybean rhizosphere mi- crobial communities found in a chronosequence of amazon soils as follow: first-year of cultivation after deforestation (1-year) to tenth (10-year) and twentieth (20-year) year of con- secutive cultivation in no-till cropping system, with succes- sive rotation of cultures, always with soybean as the main summer culture. In order to test the influence of soybean root system on modulating microbial assembly, we collected the soil samples when soybean was in the fields, at V6 vegetative stage. A total of 18 bulk soil samples were collected in January 2013, from the 0–20 cm profile. Soil samples were used to grow soybean (*Glycine max*, BRS 232 cultivar) in a greenhouse mesocosms experiment. The experiment was car- ried out with 36 vases, consisting in 18 vases with plant, to evaluate the rhizosphere effect and, 18 vases with no-plant, to evaluate the bulk soil effect (3 areas × 2 soil fractions × 6 replicates). The experiment was conducted until stage R1 (50% flowering plants), comprising the 65th day after sowing. After harvest, roots were briefly shaken to separate bulk from rhizosphere soil. The soil that remained attached to the roots was defined as rhizosphere soil and extracted from the roots with the aid of a sterile brush. Soil samples from the control vases—with no-plant—were collected and considered as bulk soil. After harvest, we collected 500 g of soil and 200 g of straw for environmental analyses. We measured or calculated 54 environmental variables, being 27 soil physicochemical attributes, 19 straw characteristics, five soil microbial enzy- matic activities and three geographical coordinates (used as constraining variables for statistical analyses). Soil and straw physicochemical analyses were performed at the Soil and Vegetal Tissue Analysis Laboratory, University of São Paulo, Piracicaba, Brazil, following routine methodology [35–39]. Soils enzymes activities were measured at the

Biogeochemistry Laboratory, São Paulo State University, Jaboticabal, Brazil, following routine methodology compiled by [40]. Detailed information regarding environmental analyses, can be found as Supplementary Material, joining the elec- tronic version of this manuscript.

DNA Extraction, Sequencing and Bioinformatics

Microbial communities were characterized through high throughput metagenomics sequencing with the Illumina MiSeq V2 kit in a MiSeq Desktop Sequencer (Illumina Inc.). DNA Sequences were processed in the Illumina MiSeq software, merged with FLASH version 1.2.11 FLASH [41] and trimmed using Phred algorithm with SeqyClean script [42]. The sequences were annotated with Metagenomics Rapid Annotation (MG-RAST) pipeline ver- sion 3.6 [43]. Taxonomic profiles were generated by matches to the M5nr Database, using best hit classification [44] while functional profiles were generated by matches to the SEED Database using hierarchical subsystems classification [45]. We used "metagenomeseq R package [46] to normalize the abundances. For details regarding DNA library preparation, sequencing procedure, and metagenome annotation see [27] (detailed information can also be found in Supplementary Material). Shotgun metagenome data are available at MG- RAST server under the project ID 7830.

Statistical Approach

To depict the variation in diversification in bulk soil and rhi- zosphere samples along the chronosequence, Sørensen beta diversity indices were calculated using the “beta.pair” func- tion (incidence-based pair-wise dissimilarities) of “betapart’” package on R software, version 3.5.1 [47]. Additionally, we performed a partitioning of Sørensen beta diversity (β_{SOR}) into the value of the turnover component, measured as Simpson dissimilarity (β_{SIM}) and the value of the nestedness component, measured as the nestedness-resultant fraction of Sørensen dissimilarity (β_{SNE}) [48]. In order to evaluate how pairwise beta diversities of neighbor communities are distrib- uted in relation to the mean β_{SOR}, we built histograms of pairwise beta diversity distributions. From each histogram output matrix, we obtained the standard deviation (SD) of each pairwise dissimilarity value of β_{SOR}. We further calculated the effect size (ES), dividing the mean value of Sørensen beta diversity (β_{SOR}) by the standard deviation of pairwise beta diversity distributions (β_{SOR}-SD) in each soil fraction and chronosequence (ES = β_{SOR}/β_{SOR}-SD). The effect size refers to the deviation of observed pairwise β_{SOR} distributions in relation to the observed value of $\beta_{SOR,}$ which indicates the distance from null expectation in a certain community.

To test whether stochastic or deterministic processes were governing microbial community assembly of the microbial

community, we calculated rank abundance distributions and immigration rates. In order to accomplish that, we used the taxonomic matrix at genus level. Species rank abundances for each metagenomic sample were fitted to five different theoret- ical assembly models: the zero-sum multinomial (ZSM) and the broken stick (null model), which regard to neutral assem- bly and the pre-emption, the log-normal and the Zipf models, related to niche-based assembly. Broken stick, pre-emption, log-normal, and Zipf models were calculated using the script "radfit" from Vegan package on R software, version 3.5.1. The ZSM model and the dispersal rates (related to dispersal process) for each sample were calculated on TeTame software [49], version 1.9. The models were compared based on the Akaike information criterion (AIC). AIC values for generated models were calculated based on the equation AIC = − 2 log- likelihood + 2 × npar, where npar represents the number of parameters in the fitted model [7, 50]. The lowest the AIC value for each sample indicates the best fitted model [51]. The dispersal rates were calculated through Etienne's formula [52].

In order to calculate the proportion of the variation in mi-
crobial assembly explained by environmental biotic and abi- otic drivers (selection process), we performed a variation partitioning of redundancy analysis (p-RDA) with principal coordinates of neighbor matrices (PCNM). The possible sin- gle and combined effects of environmental variables on the variation of microbial taxonomic assembly along the chronosequence and between bulk soil and rhizosphere were tested. In order to accomplish that, a forward selection was applied. From a set of 54 possible explanatory variables, only non-co-linear (inflation factor < 20) and significant variables ($P < 0.05$) were selected, using the Canoco software, version 5 [53]. Latitude and longitude were used as constraining spatial coordinates in the model. The resultant not explained variation from p-RDA was considered as residue + some degree of drift process.

Results

Taxonomic and Functional Profiling of Microbial Metagenomes

Shannon's α-diversity did not vary across bulk soil and rhizosphere along the chronosequence, but along time in the same soil fraction, with samples from 1-year being less ($H'_{\text{bulk-1-year}}$ = 5.2 ± 0.1; $H'_{\text{rhizosphere-1-year}}$ = 5.1 ± 0.1) di- verse than samples from 10- ($H'_{\text{bulk-10-year}}$ = 5.4 ± 0.1; $H'_{\text{rhizosphere-10-year}}$ = 5.4 ± 0.1) and 20-year ($H'_{\text{bulk-20-year}}$ = 5.4 ± 0.1; $H'_{\text{rhizosphere-20-year}}$ = 5.3 ± 0.1), with no differ-
ences between 10- and 20-year no till. Looking to taxonom- ic dynamics (Supplementary Table S1), all phyla that sig- nificantly shifted, presented lower relative abundances in

rhizosphere than in the bulk soil, along the chronosequence, except for Proteobacteria and Bacteroidetes, which had higher abundances in rhizosphere 1-year. Along the chronosequence in the bulk soil, abundances of Acidobacteria and Firmicutes significantly decreased after 10-year no-till cropping. A significant decrease on Acidobacteria and Actinobacteria abundances was ob- served after 20-year no till. Despite that, several phyla abun- dances increased in long-term no-till, such as Proteobacteria, Planctomycetes, and Gemmatimonadetes. In rhizosphere, long-term no till led phyla abundances to increase, except for Acidobacteria and Proteobacteria. In summary, main taxonomic shifts in bulk soil occurred from 1- to 10-year, while shifts in rhizosphere occurred mainly from 10- to 20-year. Depicting the variability of the phylum Proteobacteria at class level, we noticed that Alpha- and Betaproteobacteria presented higher abundances in rhizo- sphere, while Delta- and Gammaproteobacteria abundances were higher ion bulk soil. Relative abundance of Alphaproteobacteria reduced along time, while relative abundances of Beta-, Delta-, and Gammaproteobacteria in-creased, in both bulk soil and rhizosphere. Although, Alphaproteobacteria was always the most abundant Proteobacteria class, regardless time and soil fraction— 53.7% of sequences inside Proteobacteria and 23.5% of the total number of sequences — followed by Betaproteobacteria (18.8% and 8.2% respectively). We also investigated the changes in microbial functional categories along the chronosequence in bulk soil and rhizosphere (Supplementary Table S2). No significant shifts were found for any category, when comparing the relative abundances in bulk soil and rhizosphere metagenomes, but for “stress response” functional category that was higher in rhizo- sphere after 1 year. Along the chronosequence, several functional categories shifted in bulk soil, but just a few in rhizosphere. In bulk soil, the number of functional catego- ries that varied from 1 to 10 years (11) was higher than from 10 to 20 years (3), resulting in a decrease of functional cat-egories that changed due long-term no-till (10). Functional categories related to “aminoacids and derivatives” and “nu- cleosides and nucleotides” metabolism increased along the time. Meanwhile, “respiration”, “virulence, disease, and de- fense” and “potassium metabolism” decreased. In rhizo- sphere, a few functional categories shifted with long-term no till. No differences in functional categories were found from 1- to 10-year no-till. From 10 to 20 years, functions related to “respiration” and “virulence, disease, and de- fense” decreased while functions related to “clustering- based systems” increased. Overall, from 1- to 20-year chronosequence, only functions related to “potassium me- tabolism” have shifted, decreasing with long-term no till cropping. Details regarding taxonomic and functional pro-filing of microbial metagenomes can be found in [34].

Microbial Community Beta Diversity Partitioning in Bulk Soil and Rhizosphere

Overall Sørensen beta diversity was higher in rhizosphere ($\beta_{SOR} = 0.72$) than in bulk soil ($\beta_{SOR} = 0.56$) (Supplementary Fig. S1). When calculating β_{SOR} along the chronosequence (Fig. 1a), we only found differences in 1- year, where beta diversity in rhizosphere was higher ($\beta_{SOR} = 0.53$) than in bulk soil ($\beta_{SOR} = 0.29$). In 10 years (Fig. 1b), values of beta diversity were similar (bulk soil $\beta_{SOR} = 0.27$; rhizosphere $\beta_{SOR} = 0.28$), the same as found for 20-year no- till (Fig. 1c) (bulk soil $\beta_{SOR} = 0.28$; rhizosphere $\beta_{SOR} = 0.26$). We noticed that beta diversity in bulk soil did not vary along the chronosequence while it decreased in rhizosphere from 1 to 10 years, with no differences between 10- and 20-year no- till cropping. As we previously found [34] shifts in abundance for several taxa for both bulk soil and rhizosphere along the chronosequence, we depicted the Sørensen beta diversity into turnover (β_{SIM}) and nestedness-resultant dissimilarity (β_{SNE}) components, in order to explain those shifts. For bulk soil, in 1-year no-till (Fig. 1a), turnover and nestedness components of beta diversity were similar ($\beta_{SIM} = 0.14$; $\beta_{SNE} = 0.15$). In 10 years (Fig. 1b), the turnover component ($\beta_{SIM} = 0.16$) was higher than nestedness component ($\beta_{SNE} = 0.11$). Yet in 20- year no-till (Fig. 1c), the turnover was lower ($\beta_{SIM} = 0.12$) than the nestedness ($\beta_{SNE} = 0.16$). When depicting beta diver- sity in rhizosphere, we found the turnover component higher ($\beta_{SIM} = 0.31$) than the nestedness component ($\beta_{SNE} = 0.22$) in 1-year no-till. In 10 years, turnover and nestedness compo- nents were similar ($\beta_{SIM} = 0.14$; $\beta_{SNE} = 0.14$), the same as found for 20-year no-till ($\beta_{SIM} = 0.14$; $\beta_{SNE} = 0.12$). All areas had beta diversity significantly different from beta pairwise distributions (Fig. 1d–i), as shown by the high effect sizes in all cases. We also noticed that the effect sizes in rhizosphere were always higher than in bulk soil, along the chronosequence.

Community Assembly and Dispersal Rates

Since we found different patterns of beta diversities distribu- tions between bulk soil and rhizosphere communities and also along time in the same soil fractions, we sought to investigate how those observations would reflect in the outcome of theo- retical models explaining microbial assembly. In order to accomplish that, we calculated rank abundance distribution models based on Akaike information Criterion (AIC) (Table 1). In bulk soil, 14 samples fitted to zero sum model (ZSM), which is consistent with neutral theory and four to lognormal, which is consistent to niche-based theory. In rhi- zosphere, the predominant best fitted model was lognormal, with 16 samples, while two samples fitted ZSM model. There were no differences in assembly along the chronosequence in the same soil fraction. The samples of bulk soil presented, in

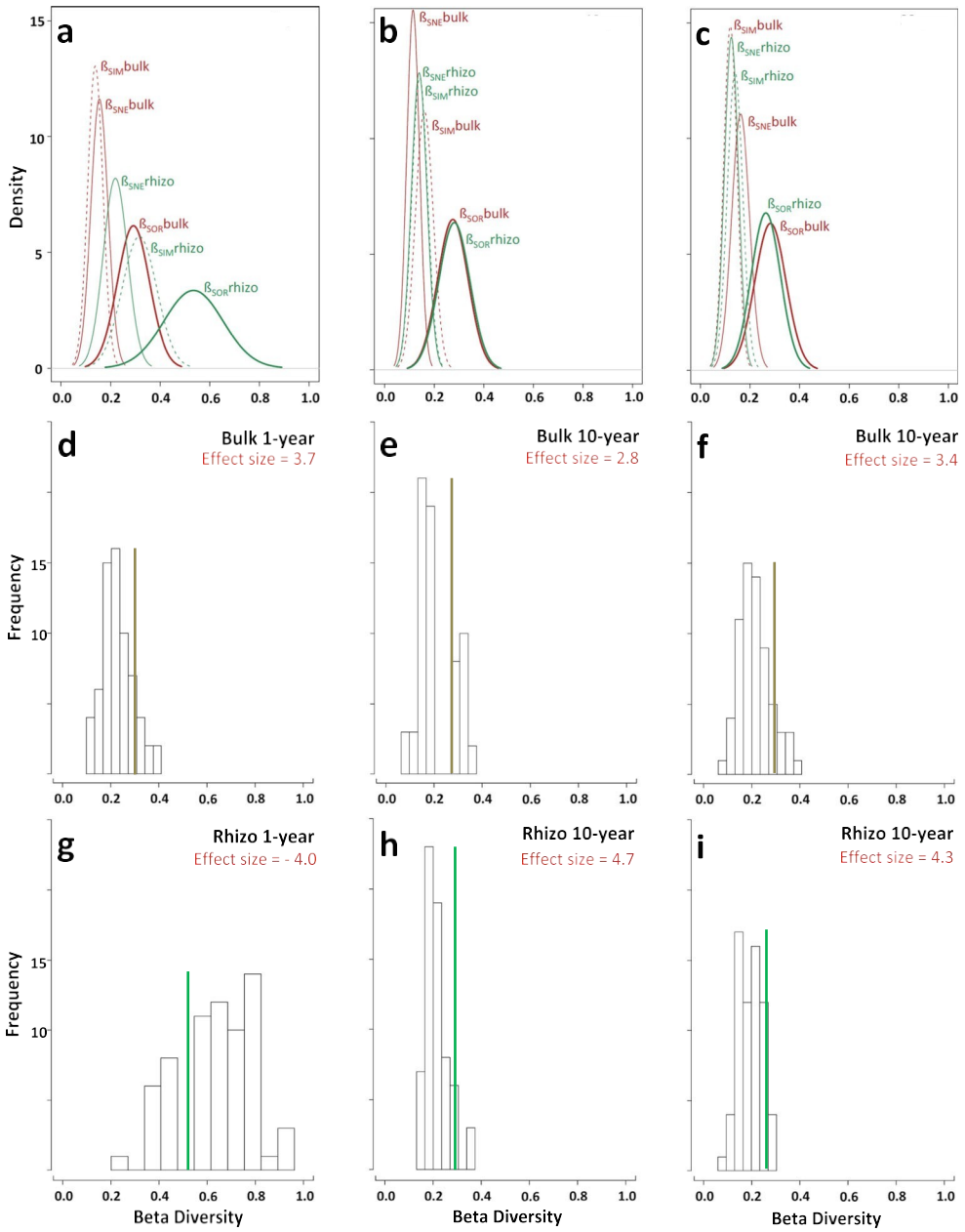

Fig. 1 Distributions of Sørensen beta diversities between microbial communities, based on taxonomic profiles at genus level (M5nr database). Distributions of observed community dissimilarities within bulk soil (brown lines) and rhizosphere (green lines) are presented as probability Kernel densities (a–c). Sørensen beta diversity distributions (β_{SOR}) is presented in bold lines. Beta diversity is decomposed in the turnover component—Simpson dissimilar- ity (β_{SIM}; thick lines) and the nestedness component (β_{SNE}; dashed lines). Histogram of ob- served community pairwise β_{SOR} distributions are presented (d–i). Histograms show the observed pairwise βSOR frequency distri- bution of microbial communities compared to the observed mean βSOR in bulk soil (brown line) and rhizosphere (green line), along the chronosequence

general, low to intermediate rates of dispersal (average = 0.41), while samples of rhizosphere presented high rates of dispersal (average = 0.69), which means more predisposition to migration from members of rhizosphere community, com- pared with those from bulk soil community.

Environmental Variation Partitioning

We performed a variation partitioning of RDA (p-RDA), gen- erated by principal coordinates of neighbor matrices (PCNM), in order to depict the role of each set of variables in structuring microbial profiles (Fig. 2), and also the contribution of selec- tion and drift (part of the residue) processes in explaining

assembly variation. In order to accomplish that, from a set of 54 variables (Supplementary Tables S3, S4, S5, and S6), we forward selected non-collinear and significant variables ($P < 0.05$) in the model. We noticed that, as no-till cropping advanced from 1- to 20-year no-till cropping, correlations be- tween physicochemical variables (abiotic factors) and taxo- nomic structures decreased in bulk soil and increased in rhi- zosphere. Differently, in the same period, correlations between straw and enzyme activities (biotic factors) with microbial assembly increased for bulk soil and decreased for rhizo- sphere. On the average of three chronosequence periods (1-, 10-, and 20-year no-till), we found more explanation of the total variation in PCNM axes of pRDA owing environmental

Table 1 Samples fitting to theoretical ecological models, based on Akaike information criterion (AIC) for rank abundance distribution at genus level, based on M5nr database. The tendency to migration was also calculated, through dispersal rate

Sample	Source	Time	Dispersal rate[b]	Akaike information criterion (AIC)[a]				
				Neutral		Niche-based		
				Broken stick	ZSM	Lognormal	Preemption	Zipf
bPA1	Bulk soil	1-year	0.33	1874.4	*1629.0*	1633.0	1782.6	1716.7
bPA2	Bulk soil	1-year	*0.46*	1701.5	*1549.2*	1562.3	1646.7	1612.6
bPB1	Bulk soil	1-year	*0.63*	2065.7	*1708.2*	1717.9	1925.8	1857.2
bPB2	Bulk soil	1-year	*0.50*	1521.8	*1450.9*	1468.8	1498.4	1478.4
bPC1	Bulk soil	1-year	*0.52*	1759.9	1599.7	*1593.8*	1694.2	1663.6
bPC2	Bulk soil	1-year	*0.60*	1561.7	*1473.3*	1490.0	1530.9	1507.8
bSA1	Bulk soil	10-year	0.33	2013.1	*1700.1*	1710.8	1880.9	1865.5
bSA2	Bulk soil	10-year	0.09	1966.6	*1698.4*	1711.9	1839.4	1862.8
bSB1	Bulk soil	10-year	*0.52*	2094.1	*1716.0*	1739.6	1937.3	1916.2
bSB2	Bulk soil	10-year	0.28	3293.2	*2011.3*	2262.0	2662.0	2767.5
bSC1	Bulk soil	10-year	0.37	1963.2	*1693.8*	1699.5	1847.5	1837.2
bSC2	Bulk soil	10-year	0.31	1902.7	1678.4	*1674.3*	1805.7	1792.4
bTA1	Bulk soil	20-year	*0.47*	2084.0	*1745.5*	1770.1	1937.3	1938.5
bTA2	Bulk soil	20-year	*0.46*	1776.0	*1607.7*	1612.8	1707.0	1699.7
bTB1	Bulk soil	20-year	0.32	1775.9	1617.5	*1605.2*	1708.1	1687.4
bTB2	Bulk soil	20-year	0.30	1966.6	*1692.4*	1694.1	1851.9	1830.1
bTC1	Bulk soil	20-year	0.36	2560.2	*1899.8*	1996.5	2250.6	2283.1
bTC2	Bulk soil	20-year	*0.53*	1631.7	1544.5	*1529.1*	1594.2	1580.6
rPA1	Rhizosphere	1-year	*1*	1452.9	1402.1	*1387.6*	1432.3	1406.1
rPA2	Rhizosphere	1-year	*1*	1402.3	1371.5	*1358.4*	1386.1	1373.9
rPB1	Rhizosphere	1-year	*1*	1648.0	1529.7	*1522.6*	1597.7	1570.6
rPB2	Rhizosphere	1-year	*1*	1176.1	1178.8	*1163.8*	1173.3	1167.1
rPC1	Rhizosphere	1-year	*1*	1230.4	1226.2	*1211.7*	1226.0	1217.1
rPC2	Rhizosphere	1-year	*1*	1264.8	1253.3	*1237.9*	1257.6	1245.0
rSA1	Rhizosphere	10-year	*0.73*	1726.3	1586.4	*1571.6*	1664.5	1649.9
rSA2	Rhizosphere	10-year	*0.49*	1979.8	*1695.9*	1704.8	1852.8	1833.4
rSB1	Rhizosphere	10-year	*0.50*	1644.0	1549.7	*1538.2*	1594.6	1594.7
rSB2	Rhizosphere	10-year	*0.78*	2023.3	1736.5	*1720.2*	1887.4	1874.8
rSC1	Rhizosphere	10-	*0.40*	2323.6	*1798*	1838.2	2096.3	2060

		year			*.3*			.9
rSC2	Rhizosphere	10-year	0.27	1889.0	1668.2	*1665.9*	1784.3	1775.2
rTA1	Rhizosphere	20-year	*0.63*	1906.0	1674.0	*1668.8*	1804.0	1790.8
rTA2	Rhizosphere	20-year	0.21	1962.0	1687.4	*1686.2*	1843.8	1813.3
rTB1	Rhizosphere	20-year	0.23	2005.2	1703.4	*1697.2*	1875.2	1847.3
rTB2	Rhizosphere	20-year	*0.85*	1728.0	1585.0	*1576.8*	1667.0	1650.4
rTC1	Rhizosphere	20-year	*0.67*	1612.4	1532.9	*1519.7*	1575.7	1566.7
rTC2	Rhizosphere	20-year	*0.79*	1596.9	1524.1	*1508.9*	1563.7	1549.0

[a] Rank abundance models based on corrected AIC value from Poisson distributions using maximum likelihood estimation. The lowest AIC value for each sample represented the best-fitted model for general community's assembly. AIC-corrected values were calculated by the Radfit function on Vegan R Package, with exception of zero-sum model distribution, which was calculated using TeTame Software. Best-fitted models were calculated by the general equation AIC = − 2log-likelihood + 2 × npar

[b] Dispersal rate were calculated by Etienne's formula, using TeTame Software. Values of dispersal are between 0 and 1, where 0 means no tendency to migration and 1 means total tendency to migration in a certain community. Values of dispersal ≥ 0.4 were highlighted in italics

For AIC, italics emphasize the best-fitted model in each metagenomic sample

ZSM zero sum multinomial

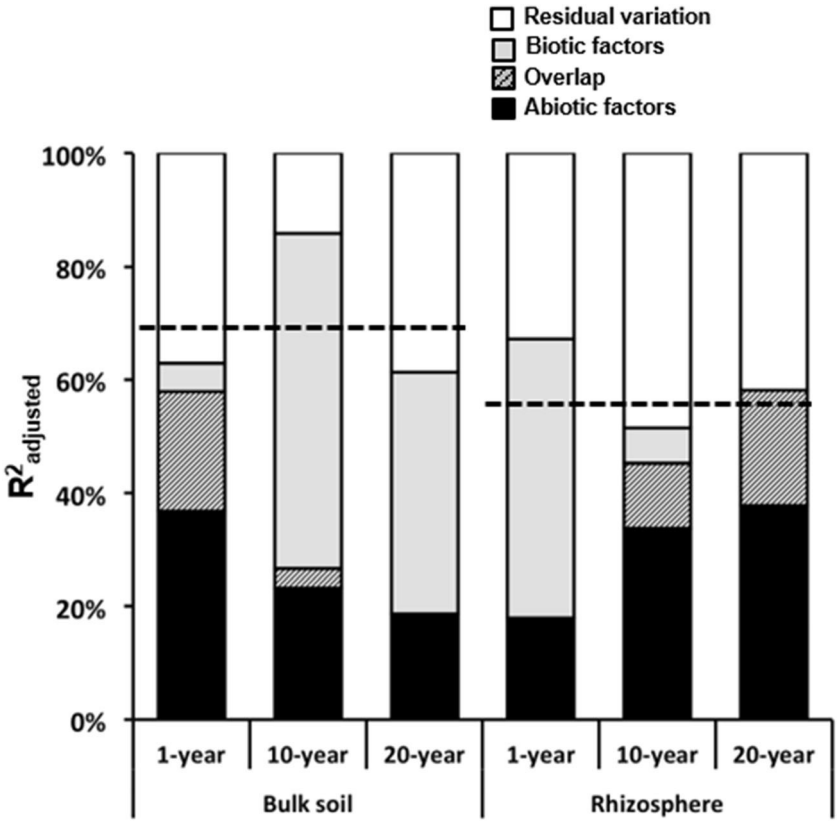

Fig. 2 Variation partitioning of redundancy analysis (pRDA) generated by principal coordi- nates of neighbor matrices (PCNM) with forward selection of explanatory variables generat- ed from Euclidean distance ma- trices, with 1000 Monte-Carlo permutations and corrected by Benjamini-Hochberg false dis- covery rate approach (FDR). Data show the adjusted coefficient of multiple determination (R^2), P adjusted ≤ 0.05, from simple ef- fects of abiotic (black) and biotic factors (light gray), and their in- teractions (overlap, dashed gray). Latitude and longitude were used as constraining spatial coordi- nates in the model

variables (abiotic + biotic) in bulk soil (R^2 $_{avera}$ = 69.8%) than in rhizosphere samples (R^2 $_{avera}$ = 57.5%). Consequently, residual variation was lower for bulk soil (R^2 $_{avera}$ = 30.2%), compared with rhizosphere ($R^2_{average}$ = 42.5%). However, the relative importance of abiotic and biotic factors differed between bulk soil and rhizosphere and also, along time for the same fraction (Table 2). In 1-year bulk soil, the variation ex- plained by abiotic factors (R^2 = 36.9%; P = 0.022) was higher than that explained by biotic factors (R^2 = 5.0%; P = 0.150). The abiotic factor that most contributed to the pRDA expla- nation was SOC. The interaction between abiotic and biotic factors explained 21% (P = 0.010) of total variation in micro- bial assembly, with no dominant explanatory variable. In 10- year bulk soil, the variation explained by abiotic factors (R^2 = 23.2%; P = 0.019) was lower than that explained by biotic factors (R^2 = 59.1%; P = 0.008). The interaction between abi- otic and biotic factors explained 3.5% of total variation (P = 0.022), with no dominant explanatory variable. The variable that most explained the abiotic variation in 10-year bulk soil was Zn, followed by longitude (constraining variable). Acid phosphatase activity was the most significant biotic variable in this group of samples. Yet in 20-year bulk soil, variation explained by abiotic factors continued to decrease (R^2 = 18.7%; P = 0.115) and not significant, while biotic factors explained 42.6%, with no overlap explanation. We found opposite patterns for rhizosphere. In 1-year rhizosphere, the variation ex- plained by abiotic factors (R^2 = 18%; P = 0.035), mainly owing to P concentration was lower than that explained by biotic factors (R^2 = 49.2%; P = 0.011), modulated by β-glucosidase activity. The interaction between abiotic and biot-

ic factors did not explain the variation in microbial structures. In rhizosphere 10-year, the variation explained by abiotic fac- tors increased (R^2 = 33.8%; P = 0.084) and was higher than that explained by biotic factors (R^2 = 6.2%; P = 0.189). The interaction between abiotic and biotic factors explained 11.5% of total variation (P = 0.059). Yet in 20-year rhizosphere, var- iation explained by abiotic factors continued to increase, reaching 37.8% (P = 0.021), with overlap explaining 20.4% (P = 0.033), while no explanation due biotic factors were found.

Discussion

Soil microorganisms are a key component of natural and man- aged ecosystems [54]. Microbial community ecology can help us to predict scenarios of long-term agriculture exploration in deforested areas, influencing practices for sustainable food production and biodiversity conservation. This study emerged in order to advance our previous understanding about micro- bial assembly in long-term agricultural cropping systems, after deforestation of Brazilian tropical forests, with emphasis to the Amazon Rainforest [1–3, 6]. To accomplish that, we eval- uated microbial patterns and ecological process [14, 55]

Table 2 Correlation between microbial phyla and environmental variables, along the chronosequence and soil fractions. Values extracted from the PCNM of pRDA analysis

Area	Factors	*F*-value	*P* value	Variables	Positive (+) or negative (−) correlation with taxonomic groups at phylum or class* level
Bulk soil					
1-year	Abiotic	5.2	0.022	SOC	Actinobacteria (+), α-Proteobacteria (−), γ-Proteobacteria (−), Firmicutes (−)
	Overlap	4.6	0.010		
	Biotic	2.8	0.150	β-glucosidase	α-Proteobacteria (+), γ-Proteobacteria (+), δ-Proteobacteria (+), Actinobacteria (−)
10-year	Abiotic	11.1	0.019	Zn, longitude	α-Proteobacteria (+), β-Proteobacteria (+), γ-Proteobacteria (+), Actinobacteria (−)
	Overlap	12.7	0.022		
	Biotic	9.4	0.008	Acid phosphatase	Actinobacteria (+), α-Proteobacteria (−), β-Proteobacteria (−), γ-Proteobacteria (−), δ-Proteobacteria (−)
20-year	Abiotic	3.6	0.115	Moisture	Firmicutes (+), Actinobacteria (+), α-Proteobacteria (−)
	Overlap	–	–	–	–
	Biotic	4.6	0.066	–	α-Proteobacteria (+), β-Proteobacteria (+), Verrucomicrobia (+), δ-Proteobacteria (+), Actinobacteria (−), Firmicutes (−)
Rhizosphere					
1-year	Abiotic	4.3	0.035	P	δ-Proteobacteria (+), Planctomycetes (+), Actinobacteria (−)
	Overlap	–	–	–	–
	Biotic	5.7	0.011	β-glucosidase	δ-Proteobacteria (+), Acidobacteria (+), Firmicutes (+), β-Proteobacteria (−)
10-year	Abiotic	3.7	0.084	Mg^{2+}	Actinobacteria (+), δ-Proteobacteria (−)
	Overlap	4.3	0.059		
	Biotic	2.1	0.189	Dehydrogenase	β-Proteobacteria (−)
20-year	Abiotic	4	0.021	$N\text{-}NO_3^-$	Actinobacteria (+), α-Proteobacteria (+), Cyanobacteria (+), β-Proteobacteria (−), γ-Proteobacteria (−), Acidobacteria (−),

*Members of the phylum Proteobacteria are presented here at the class level

modulating soil microbial assembly, in a long-term forest-to- agriculture conversion chronosequence, in soybean fields at the same toposequence, in Eastern Amazon.

Several studies have applied huge effort, in order to depict assembly patterns and the ecological processes governing them, in macroecology [55, 56] and microbial ecology [14, 57]. However, there is no concern about how assembly models can predict the interaction of ecological processes governing taxa abundance distribution in different ecosystems, along time and/or space [58–60]. Since we found an evident microbial diversity loss after deforestation and long- term agriculture exploitation, we sought to depict the ecological outcome of anthropogenic action on that chronological sequence. Thus, the aim of this work was to evaluate the ecological processes governing microbial assembly, in bulk

soil and soybean rhizosphere, along a long-term forest-to- agriculture conversion chronosequence, in Eastern Amazon.

We hypothesized that weight of the four ecological process- es governing assembly differs between bulk soil and rhizo- sphere and along the chronosequence in the same fraction. In a microbial metagenomics study, evaluating assembly in bulk soil and rhizosphere of soybean, in an amazon's no-till cropping system, authors found the same assembly patterns [7]. Our analysis showed that most bulk soil samples fitted a neutral-based model (ZSM), regarding stochastic assembly processes [61] while rhizosphere samples fitted a niche-based model (lognormal), related to deterministic assembly processes [62]. This observation led us to partially reject our first hypoth- esis in which we stated that assembly models could vary with time in the same soil fraction, which was found not true, since

most of the samples in bulk soil fitted a neutral-based model while most of the rhizosphere samples fitted a niche-based model, regardless time. Nestedness of species assemblages is expected to predominate when sites with lower number of species are subsets of sites with higher richness, which leads to diversity loss due to any factor that constantly promote assemblage disturbance [48], as found for bulk soil in 1- and 20-year. In the other hand, taxa turnover occurs when some species are replaced by others, as consequence of spatial his- torical contingency [63] or environmental sorting [29] as evi- denced in 1- and 20-year rhizosphere and 10-year bulk soil. All areas had beta diversity significantly different from beta pairwise distributions, as shown by the high effect sizes in all cases that could lead to the assumption that all communities would follow a deterministic assembly [32]. We also noticed that the effect sizes in rhizosphere were always higher than in bulk soil, along the chronosequence, indicating that the distur- bance of soil forest-to-agriculture conversion was more pro- nounced on that fraction than in bulk soils [64].

As deterministic diversification (Fig. 1) and stochastic neu- tral model (Table 1) are likely to explain assembly in bulk soils, we sought to investigate evidences of the governing processes in this soil fraction. It is assumed by microbial com- munity ecologists that, when assembly modeling follows stochasticity, the influence of selection tends to be low [14, 25]. Moreover, a link between ZSM neutral-based model and dispersal limitation is regarded as a central mechanism explaining stochastic community abundances distribution [65]. Our results corroborated this ecological trend since, coupled to predominantly ZSM assembly in bulk soil, we found low to moderate dispersal rates (average = 0.41), indi- cating a role of dispersal limitation processes on microbial assembly patterns. Dispersal limitation is a stochastic process consistent with transient abundance in microbial communi- ties. Transient abundant taxa are the ones that immigrated to the community or emerged in a certain environment, due to diversification [63]. In some cases, those species can be ex- tinct as a result of drift and/ or dispersal limitation. In addition, as the chronosequence advanced, the variation in bulk soil microbial assembly explained by abiotic factors (homoge- neous selection process) became lower, ranging from 36.9% in 1-year to 23.2% in 10-year, reaching a minimum 18.7% in 20-year no-till. Despite that, we found that biotic factors, cor- responding to variable selection process, a deterministic pro- cess, increased their role in explaining assembly variation, as the chronosequence advanced, ranging from 5% in 1-year no- till cropping to a maximum of 59.1% in 10-year no-till cropping and, 42.6% in 20-year no-till cropping. Variable se- lection is a deterministic process [18], linked to conditional abundances in microbial communities. In this case, species are conditionally regulated, with some reaching high abundances in the community while others fluctuate, driven by variable selection. Coupled stochasticity and deterministic processes

can result in two possible scenarios. (1) If the role of dispersal limitation is linked to changes in diversification and drift, we can deduce that communities are in a transient abundance state, which is likely to occur when immigration history has a marked impact due to dispersal limitation [63]. (2) If there is no dispersal limitation, the role of variable selection is pro- nounced, leading communities to have abundances condition- ally regulated [66]. In this case communities are likely to be in conditional abundance state, in which some members of the community have fluctuations in abundance along time. The communities in bulk soil had assembly and patterns that cul- minated in a mix of both scenarios, leading to the assumption that the turnover of communities in bulk soil is governed by undominated processes [58], with characteristics of dispersal limitation due to spatial contingency and variable selection processes.

Most of the attempts to underlie plant microbiome selection show a clear selection of plant root system [7, 67]. Besides, some evidences of selection according to plant genotype and cultivar are mentioned [24, 68]. The predominantly niche- based assembly, found for soybean rhizosphere communities, is often consistent with the deterministic ecological process of selection [7]. Moreover, the high effect sizes and deviation from null beta diversity distributions (Fig. 1) confirm the ten- dency of rhizosphere communities to be governed by deter- ministic mechanisms. Microbial ecologists have linked deter- minism with pressures imposed by the environment, regulat- ing taxa trade-offs through homogenizing or variable selection [18, 27]. When homogenizing selection is acting, we expect the role of abiotic factors modulating assembly to be high. In this cases, several works have observed that, homogenizing selection, act in order to constrain microbial diversity along time and/or space, leading to biotic homogenization and loss of diversity [1, 31]. Several studies show that the community composition is strongly related to land use [27, 69] and envi- ronmental conditions [17, 70–73], which is indicative of ho- mogeneous selection. Otherwise, whether variable selection is predominant, biotic factors are more likely to explain micro- bial assembly patterns [18, 25]. We found that the variation of microbial assembly in soybean rhizosphere explained by abi- otic factors increased, as the chronosequence advanced, rang- ing from 18% in 1-year no till to 33.8% in 10-year no till, reaching a maximum 37.8% in 20-year no-till. Those results indicated an increasing role of homogeneous selection process on modulating microbial assembly in soybean rhizosphere, as the chronosequence advanced. Unlikely, biotic factors ex- plained 49.2% of the total variation in 1-year no till, 6.2% in 10-year no till, with no explanation by biotic factors in 20-year no-till due these factors in 20-year no-till cropping. Furthermore, when looking to dispersal rates, we found that the tendency of migration by members of the communities in rhizosphere was high (average = 0.69). This average is mainly driven by a dominant homogenizing dispersal, found in

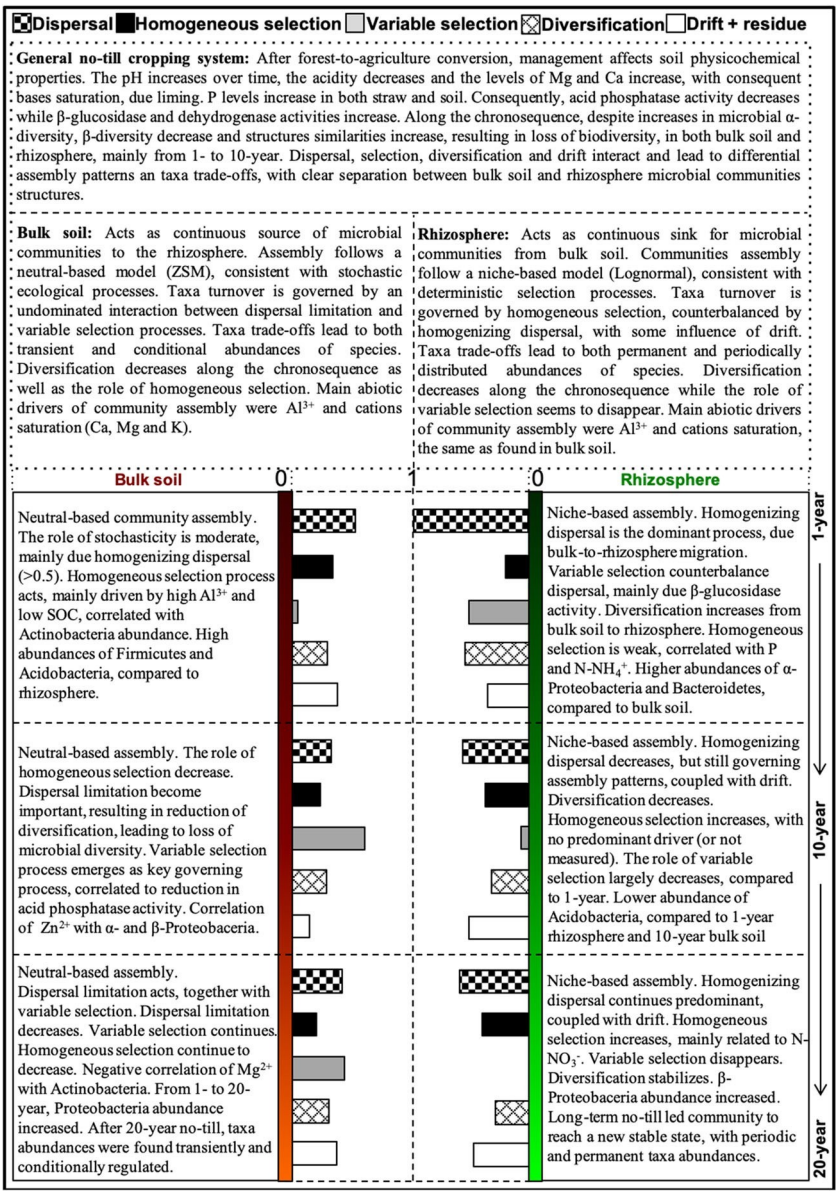

Fig. 3 Framework summarizing key patterns ecological processes, dispersal, selection, speciation, and drift. Selection is divided in two directions: homogeneous selection (pressure by abiotic factors) and variable selection (pressure by biotic factors). Bars show single effects of the four ecological processes governing assembly in bulk soil (from dark to light brown) and soybean rhizosphere (from dark to light green), along an 1- to 20-year chronosequence. Interaction be- tween processes and patterns are discussed on panels. Bars of dis- persal rates were obtained by Etienne's formula. Bars of selec- tion and drift were obtained by variation partitioning of redun- dancy analysis (pRDA), generat- ed by principal coordinates of neighbor matrices (PCNM), with forward selection and FDR cor- rection. Bars of diversification were obtained through pairwise Sørensen beta diversity distribu- tions. Adapted from [25]

soybean rhizosphere in early successional stage (1-year no- till; average = 1). Depicting the role of dispersal, along the chronosequence, we found moderate dispersal rates in both 10-year no till (average = 0.53) and 20-year no-till (average = 0.56). Thus, we can deduce that, homogenizing dispersal was the pivotal ecological process governing soybean rhizosphere assembly in 1-year no-till. For 10-year chronosequence, ho- mogeneous selection process became evident, allied to ho- mogenizing dispersal. After 20-year no-till cropping, the role of homogeneous selection continued to increase, coupled with moderate homogenizing dispersal. It is been suggested by theoretical ecologists that, after some transient period, which is dependent on immigration history and counterbalance of biotic (variable selection) and abiotic pressures (homogeneous selection) [74], communities can reach a new stable state [63,

75]. This new stable state can lead taxa turnover to be similar to that found before disturbance, as observed in successional stages of forest communities. Unlikely sometimes, after dis- turbance, communities reach a new stable state with different taxa turnover patterns, compared with those before distur- bance. Then, one can deduce that communities can reach al- ternative stable states or even multiple stable states [5]. Community's abundances are prone to remain in dynamic turnover equilibrium in two main scenarios: (1) Stochastic equilibrium, where homogenizing dispersal process governs community assembly, with drift as possible second determi- nant of abundances regulation. In this case community abun- dances trade-offs, follow a periodic trade-off state or even permanent distribution state. (2) Deterministic equilibrium, where homogeneous selection, through environmental abiotic

factors act, in order to lead abundances to a permanent stable state. After long-term no-till, we found community assembly in soybean rhizosphere regulated by both dispersal and homo- geneous selection. This coupled governing processes lead taxa abundances to present a mix of periodic and permanent distribution, which characterizes a new stable state, derived from long-term no-till cropping. Once reached this new abundance stable state, with established governing processes modulating assembly, abundance trade-offs tend to remain in that periodic or permanent distribution state, unless a new dramatic distur- bance event occurs [63].

Few studies have evaluated and discussed how ecolog- ical processes modulate the microbial communities along time [26] and more specifically, how these processes interact to explain microbial trade-offs in the soil-rhizosphere interface [7, 76]. As an outcome of ecological processes governing differential assembly across the bulk soil- rhizosphere interface, we proposed a framework summarizing the key insights in terms of assembly patterns, eco- logical processes, and possible environmental features im- posing them (Fig. 3). To conceive those interpretations, we followed the conceptual model described by [55], with implementations for microbial ecology [25].

Conclusions

Forest-to-agriculture conversion generally culminates with loss of biodiversity. Knowing that, microbial ecologists had for long examined microbial community diversity, structure, and composition, but studies depicting the role of ecological processes governing those assembly patterns and conse- quences for ecosystems function in natural and managed eco- systems are scarce. Moreover, the role of the plant root system in modulating microbial assembly and ecological processes in agroecosystems is often neglected. Here, we used a metagenomics approach to link patterns and processes in a more comprehensive way. We demonstrated that, despite long-term no-till lead to losses in microbial diversity in both bulk soil and soybean rhizosphere, community assembly and ecological processes varied across soil fractions. Assembly in bulk soil was predominantly neutral-based while in soybean rhizosphere most of the samples followed a niche-based mod- el, regardless time of conversion from forest to no-till cropping system. In bulk soil, microbial assembly was governed by undominated ecological processes of stochastic dispersal limitation and deterministic variable selection. Consequently, taxa turnover, after long-term no-till was found transiently and conditionally regulated by the combination of those processes. Yet for soybean rhizosphere, assembly was governed predominantly by homogenizing dispersal coupled with increased homogeneous selection, as the chronosequence advanced. After long-term no-till, those coupled governing

processes lead taxa abundances to present a mix of periodic and permanent distribution, which characterizes a new stable state, derived from long-term no-till cropping. Additionally, increased homogenous selection evidenced the power of soy- bean root system in modulating taxa-trade-offs. Our study provides a more comprehensive picture of the relationships between microbial patterns and the ecological processes mod- ulating them. More than that emphasizes plant-microbiome interactions and the possible consequences for ecosystem ser- vices and stability. Further studies could address the cause/ effect relationship along the plant-rhizosphere-soil continuum, in order to elucidate whether the plant selects its microbiome according to functions or the soil pressures the plant to select taxa, via source/ sink gradient. Thus, deciphering the ecologi- cal processes regulating plant-microbiome assembly and the causality nexus across the plant-microbiome-soil continuum may enable researchers to gain insights about plant bioengi- neering and soil microbiome modulation, with consequences for clean food production and ecosystem services resiliency.

Author Contributions DG-S and SMT designed the project. DG-S col- lected the soil samples. DG-S conducted the experiment. DG-S and LWM performed the metagenome analyses. DG-S and LWM analyzed the meta- data. DG-S, LWM, JLMR, and SMT wrote the manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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